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Effects of acute hypercapnia with and without acidosis on lung inflammation and apoptosis in experimental acute lung injury

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ABSTRACT

We investigated the effects of acute hypercapnic acidosis and buffered hypercapnia on lung inflammation and apoptosis in experimental acute lung injury (ALI). Twenty-four hours after paraquat injection, 28 Wistar rats were randomized into four groups ($n = 7$/group): (1) normocapnia (NC, $PaCO_2 = 35–45$ mmHg), ventilated with $0.03\%CO_2 + 21\%O_2 +$ balanced N_2; (2) hypercapnic acidosis (HC, $PaCO_2 = 60–70$ mmHg), ventilated with $5\%CO_2 + 21\%O_2 +$ balanced N_2; and (3) buffered hypercapnic acidosis (BHC), ventilated with $5\%CO_2 + 21\%O_2 +$ balanced N_2 and treated with sodium bicarbonate (8.4%). The remaining seven animals were not mechanically ventilated (NV). The mRNA expression of interleukin (IL)-6 ($p = 0.003$), IL-1β ($p < 0.001$), and type III procollagen (PCIII) ($p = 0.001$) in lung tissue was more reduced in the HC group in comparison with NC, with no significant differences between HC and BHC. Lung and kidney cell apoptosis was reduced in HC and BHC in comparison with NC and NV. In conclusion, in this experimental ALI model, hypercapnia, regardless of acidosis, reduced lung inflammation and lung and kidney cell apoptosis.

1. Introduction

Protective ventilation with low tidal volume has been shown to improve survival in patients with acute respiratory distress syndrome (ARDS) (ARDSNetwork, 2000; Ware and Matthay, 2000; Putensen et al., 2009), but may be associated with increased arterial carbon dioxide levels, generating hypercapnic acidosis. Acute hypercapnic acidosis has been suggested to attenuate lung injury in ischemic reperfusion (Laffey et al., 2000a), sepsis (Costello et al., 2009; Higgins et al., 2009), and endotoxin (Norozian et al., 2011) models, raising the possibility of a potential therapeutic application of hypercapnia in acute lung injury (ALI) (therapeutic hypercapnia).

It is still unclear whether acidosis should be buffered in therapeutic hypercapnia to reduce the systemic side effects associated with low arterial pH (pHa) (Bautista and Akca, 2013). Buffering acute hypercapnic acidosis with bicarbonate infusions was permitted in the ARDS Network tidal volume study (Brower et al., 2004). In experimental ALI induced by pulmonary ischemia–reperfusion (Laffey et al., 2000a) or cecal ligation and puncture (Higgins et al., 2009), buffering reduced the protective effects of hypercapnic acidosis, but worsened lung damage in *E. coli*-induced ALI (Nichol et al., 2009).

Hypercapnic acidosis affects not only lung inflammation, but may influence the apoptosis process as well (Shibata et al., 1998). Since CO_2 is highly diffusible through biologic tissues, it may modulate apoptosis not only in the lung but also in peripheral organs. To our knowledge, no study has evaluated, in experimental ALI, whether buffering acute hypercapnic acidosis may dampen cell apoptosis in different organs.

We hypothesized that hypercapnia with acidosis would reduce lung inflammation and apoptosis in lung and distal organs, whereas buffering may decrease these beneficial effects. The aim of this

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study was to investigate the effects of acute hypercapnia with and without acidosis on lung inflammation and lung, kidney, and liver cell apoptosis in paraquat-induced ALI.

2. Methods

2.1. Animal preparation and experimental protocol

This study was approved by the Ethics Committee of the institution where the work was carried out. All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the U.S. National Academy of Sciences.

In 28 Wistar rats (weight 250–300 g, age 8 weeks), paraquat was administered (15 mg/kg by intraperitoneal injection). After 24 h, animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and midazolam (5 mg/kg). An intravenous line (Jelco 24G) was placed in the tail vein and anesthesia was maintained intravenously with ketamine (50 mg/kg/h) and midazolam (2.5 mg/kg/h). A comparable amount of sedative and anesthetic drugs was given to all rats. The depth of anesthesia was similar in all animals. Animals were placed and kept in supine position throughout the whole experiment. After median neck incision, a polyethylene catheter (PE 10) was introduced into the right internal carotid artery for blood sampling and mean arterial blood pressure (MAP) measurement (SCIREQ, Montreal, Canada). Under spontaneous breathing of room air (T0), blood (300 μL) was drawn into a heparinized syringe for measurement of arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), pHa, and bicarbonate (HCO_3^-) (i-STAT, Abbott Laboratories, Abbott Park, IL, USA). Animals were then paralyzed (pancuronium bromide 2 mg/kg intravenously) and mechanically ventilated (Servo-i, MAQUET, Sweden) in volume-controlled ventilation with tidal volume (V_T) = 8 ml/kg, respiratory rate (RR) = 80 breaths/min, inspiratory/expiratory ratio = 1:2, fraction of inspired oxygen (FiO_2) = 0.21, and positive end-expiratory pressure (PEEP) = 5 cmH_2O. Animals were randomly allocated to three groups (n = 7 per group): (1) normocapnia (NC) – mechanically ventilated as described above to maintain $PaCO_2$ between 35 and 45 mmHg; (2) hypercapnia (HC) – mechanically ventilated with a gas mixture containing 5% CO_2, 21% O_2, and balanced N_2 (Linde Gas Therapeutics, Lidingö, Sweden) to maintain $PaCO_2$ between 60 and 70 mmHg; and (3) buffered hypercapnia (BHC) – mechanically ventilated with a gas mixture containing 5% CO_2, 21% O_2, and balanced N_2 (Linde Gas Therapeutics, Lidingö, Sweden) to maintain $PaCO_2$ between 60 and 70 mmHg. After 5 min, a bolus of sodium bicarbonate solution (8.4%) was given intravenously equivalent to one-third of the dose calculated according to the following equation: HCO_3^- (mEq) = 0.3 × weight (kg) × base excess (BE) (Sirieix et al., 1997). The remaining seven animals were not mechanically ventilated (NV) and were used for measurement of lung histology and molecular biology analysis (Fig. 1). Arterial blood gases were analyzed at 0 (T0), 10 (T10) and 60 (T60) min after randomization.

After 60 min of mechanical ventilation, animals were euthanized and lungs and distal organs prepared for histological examination and molecular biology analysis.

2.2. Lung histology

At 60 min, a laparotomy was performed and heparin (1000 IU) injected into the vena cava. The trachea was clamped at end-expiration (PEEP = 5 cmH_2O), and the abdominal aorta and vena cava were severed, yielding massive death by exsanguination. The right lung of each animal was quick-frozen by immersion in liquid nitrogen, fixed with Carnoy's solution, and embedded in paraffin (Nagase et al., 1992). Four-micrometer-thick slices were cut and stained with hematoxylin-eosin. Morphometric analysis was performed with an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines of known length coupled to a conventional light microscope (Olympus BX51, Olympus Latin America-Inc., Brazil). The volume fraction of the lung occupied by collapsed alveoli (alveoli with rough or plicate walls) or normal pulmonary areas (those not exhibiting overdistended or plicate walls) were determined by the point-counting technique (Weibel, 1990) at a magnification of ×200 across 10 random, non-coincident microscopic fields (Riva et al., 2008; Saddy et al., 2010). Neutrophils, mononuclear leukocytes, and total cells in the lung parenchyma were evaluated at ×1000 magnification and determined by the point-counting technique. Diffuse alveolar damage (DAD) was quantified using a weighted scoring system (Silva et al., 2013). Briefly, values from 0 to 4 were used to represent the severity of edema, hemorrhage, inflammatory infiltration, and alveolar collapse, with 0 standing for no effect and 4 for maximum severity. Additionally, the extent of each parameter per field of view was scored on a scale of 0 to 4, with 0 standing for no appearance and 4 for complete involvement. Scores were calculated as the product of severity and extent of each feature, and ranged from 0 to 16. The cumulated DAD score was calculated as the sum of single score characteristics, yielding scores from 0 to 64. Two investigators blinded to the origin of the material examined the samples microscopically.

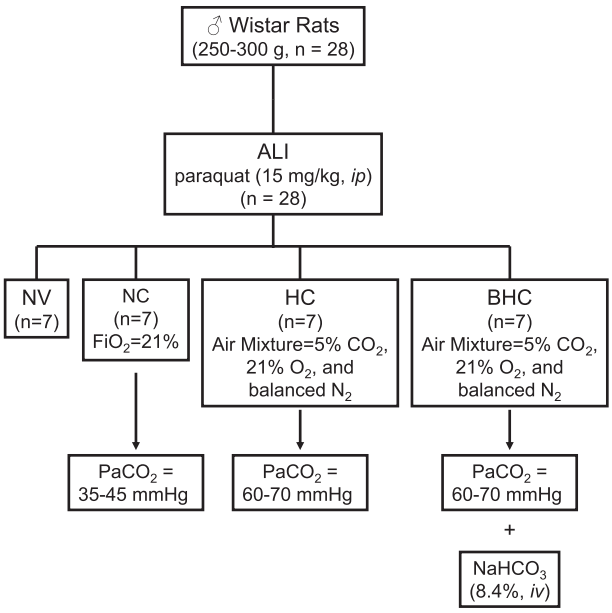

Fig. 1. Schematic flow chart of the study design. Acute lung injury (ALI) groups were randomized as follows: (a) normocapnia (NC), (b) hypercapnia (HC), and (c) buffered hypercapnia (BHC). For lung morphometry and molecular biology analysis, an additional non-ventilated (NV) ALI group was used.

2.3. Apoptosis assay of lungs, kidneys, and liver

Left lungs, kidneys, and liver were removed, fixed in 4% buffered formalin, and embedded in paraffin. To assay cellular apoptosis, terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) staining was performed by two pathologists unaware of group allocation. Apoptotic cells were detected using an In Situ Cell Death Detection Kit, Fluorescein (Boehringer, Mannheim, Germany). Nuclei without DNA fragmentation stained blue as a result of counterstaining with hematoxylin (Oliveira et al., 2009;

Steimback et al., 2009). Ten fields per section from regions with apoptotic cells were examined at a magnification of ×400. A five-point, semi-quantitative, severity-based scoring system was used to assess the degree of apoptosis in lung, kidney, and liver, graded as follows: 0, normal parenchyma; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100% of examined tissue.

2.4. IL-6, IL-1β, and PCIII mRNA expression

Quantitative real-time reverse transcription (RT) polymerase chain reaction (PCR) was performed to measure the relative levels of IL-6, IL-1β, and PCIII gene expression in lung tissue. Central slices of right lung were cut, collected in cryotubes, flash-frozen by immersion in liquid nitrogen, and stored at −80 °C. Total RNA was extracted from the frozen tissues using Trizol reagent (Invitrogen, Carlsbad, CA). RNA concentration was measured by spectrophotometry in a Nanodrop® ND-1000 system. First-strand cDNA was synthesized from total RNA using an M-MLV Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA). PCR primers for the target gene were purchased (Invitrogen, Carlsbad, CA). The following primers were used: IL-6 (sense 5′-CTC CGC AAG AGA CTT CCA G-3′ and antisense 5′-CTC CTC TCC GGA CTT GTG A-3′); IL-1β (sense 5′-CTA TGT CTT GCC CGT GGA G-3′ and antisense 5′-CAT CAT CCC ACG AGT CAC A-3′); PCIII (sense 5′-ACC TGG ACC ACA AGG ACA C-3′ and antisense 5′-TGG ACC CAT TTC ACC TTT C-3′); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sense 5′-GGT GAA GGT CGG TGTG AAC-3′ and antisense 5′-CGT TGA TGG CAA CAA TGT C-3′). Relative mRNA levels were measured with a SYBR green detection system using ABI 7500 Real-Time PCR (Applied Biosystems, Foster City, CA). All samples were measured in triplicate. The relative expression of each gene was calculated as a ratio compared with the reference gene, GAPDH, and expressed as fold change relative to NV animals.

2.5. Statistical analysis

Normality of data was tested using the Kolmogorov–Smirnov test with Lilliefors' correction, while the Levene median test was used to evaluate the homogeneity of variances. If both conditions were satisfied, one-way ANOVA was used. To compare nonparametric data, one-way ANOVA on ranks followed by the Dunn *post hoc* test was selected. The effects of time course on gas analysis were determined by one-way repeated-measures ANOVA followed by the Tukey test. Parametric data were expressed as mean ± SD and nonparametric data were expressed as median (interquartile range). All tests were performed using the Sigma Plot 11.0 statistical software package (Jandel Corporation, San Raphael, CA, USA). Statistical significance was established as $p < 0.05$.

Table 1
Blood gas analysis.

	NC	HC	BHC
$PaCO_2$ (mmHg)			
T0	45.9 ± 0.9	45.1 ± 1.3	43.8 ± 0.9
T10	46.7 ± 0.7	68.8 ± 1.8**	65.6 ± 0.7**
T60	43.2 ± 2.7	72.9 ± 1.5**	65.6 ± 1.1**,#
pHa			
T0	7.32 ± 0.01	7.29 ± 0.01	7.33 ± 0.02
T10	7.29 ± 0.01	7.21 ± 0.01**	7.31 ± 0.01
T60	7.35 ± 0.03	7.21 ± 0.01**	7.32 ± 0.01
HCO_3 (mmol/L)			
T0	22.92 ± 0.62	20.97 ± 0.27	22.43 ± 0.48
T10	21.80 ± 0.61	26.32 ± 0.48**	32.48 ± 0.34**,#
T60	23.27 ± 1.10	28.37 ± 0.48**	33.10 ± 0.65**,#
PaO_2 (mmHg)			
T0	57.7 ± 2.29	57.98 ± 2.37	59.18 ± 2.82
T10	77.9 ± 2.62	79.78 ± 1.24	82.05 ± 1.14
T60	89.62 ± 2.45	88.06 ± 2.18	91.21 ± 2.60

Values expressed as mean (±SD) of 7 rats in each group. $PaCO_2$, pHa, HCO_3^- and PaO_2 at baseline (T0), after administration of CO_2 (T10), and at 60 min (T60). Paraquat-induced ALI animals were randomized into normocapnia (NC), hypercapnia (HC), and buffered hypercapnia (BHC) groups.
** Significantly different from NC ($p < 0.05$).
# Significantly different from HC ($p < 0.05$).

3. Results

In all experimental groups, mean arterial pressure (MAP) remained stable (higher than 70 mmHg) during the 60 min of mechanical ventilation.

In the HC group, $PaCO_2$ increased from T0 to T10 and then stabilized between 60 and 70 mmHg while pHa decreased. In the BHC group, pHa returned to values similar to NC at T10, whereas HCO_3^- was increased compared to HC and NC. There was no difference in PaO_2 among groups (Table 1).

The fractional area of collapsed alveoli and neutrophils was lower in the HC and BHC groups than in the NC and NV groups (Table 2). DAD score values were reduced in HC compared to NC and NV groups (Table 3) Edema and hemorrhage did not differ among

Table 2
Lung morphometry.

	Normal (%)	Alveolar collapse (%)	Total cells (%)	Neutrophil (%)	MN (%)
NV	69.5 ± 3.6	30.5 ± 3.6	50.1 ± 3.2	14.6 ± 3.5	35.4 ± 3.6
NC	70.1 ± 5.5	29.9 ± 5.5	48.9 ± 3.8	14.8 ± 2.3	35.2 ± 1.3
HC	85.1 ± 4.2**	14.9 ± 4.2**	44.5 ± 4.1**	9.4 ± 1.0**	35.1 ± 3.2
BHC	85.0 ± 3.6**	15.0 ± 3.6**	41.8 ± 0.9**	8.7 ± 0.7**	33.0 ± 0.7

Values expressed as mean (±SD) of 7 rats in each group. Ten random, noncoincident microscopic fields were analyzed in each lung. MN, mononuclear cells. Paraquat-induced ALI animals randomized into non-ventilated (NV), normocapnia (NC), hypercapnia (HC), and buffered hypercapnia (BHC) groups.
** Significantly different from NC ($p < 0.05$).

Table 3
Variables of diffuse alveolar damage.

	Edema	Hemorrhage	Inflammatory infiltration	Alveolar collapse	Cumulated DAD score
NV	2 (1.5–2.5)	0 (0–1)	6 (4–6)	4 (3–5)	12 (9–14)
NC	2 (1.5–3)	1 (0–1)	4(4–6)	4 (3–6)	12 (10–14)
HC	2 (1.5–2)	0 (0–2)	2 (2–3)*	1 (1–2)*,**	6 (5.5–7.5)*,**
BHC	2 (1.5–2)	2 (1.5–2)	2 (1.5–2.5)*,**	2 (1–2.5)	7 (6–9)

Values are expressed as median (interquartile range) of 7 animals in each group. Paraquat-induced ALI animals randomized into non-ventilated (NV), normocapnia (NC), hypercapnia (HC), and buffered hypercapnia (BHC) groups.
* Significantly different from NV ($p < 0.05$).
** Significantly different from NC ($p < 0.05$).

Table 4
Cell apoptosis in lung, kidney, and liver.

	Lung	Kidney	Liver
NV	3 (2.75–4)	3 (3–4)	3 (2–3.25)
NC	3 (3–4)	3 (3–3.25)	3 (2–3)
HC	2 (1–2)**	2 (1–2)**	2 (2–3)
BHC	2 (1–2)**	2 (1–2)**	2 (2–3)

Values expressed as median (interquartile range) of 5 animals in each group. A 5-point, semi-quantitative, severity-based scoring system was used. Apoptotic findings were graded as follows: 0, normal lung parenchyma; 1, 1–25% of examined tissue; 2, 26–50% of examined tissue; 3, 51–75% of examined tissue; 4, 76–100% of examined tissue. Paraquat-induced ALI animals randomized into non-ventilated (NV), normocapnia (NC), hypercapnia (HC), and buffered hypercapnia (BHC) groups.
** Significantly different from NC ($p < 0.05$).

groups. Alveolar collapse was more reduced in HC compared to NC and NV. Inflammatory infiltration was decreased in HC and BHC (Table 3).

The degree of lung and kidney cell apoptosis was more reduced in the HC and BHC groups than in NC and NV. However, the degree of liver cell apoptosis was similar in all groups (Table 4).

IL-6, IL-1β, and PCIII expression in lung tissue was lower in HC than NC and NV. IL-6, IL-1β, and PCIII expression was similar in the HC and BHC groups. PCIII expression was higher in NC compared to NV (Fig. 2).

4. Discussion

In paraquat-induced ALI, we found that therapeutic hypercapnia with and without acidosis reduced alveolar collapse, lung inflammatory response, diffuse alveolar damage, expression of type III procollagen, and degree of lung and kidney cell apoptosis. To our knowledge, this study is among the first to compare the effects of acute hypercapnic acidosis and buffering of hypercapnic acidosis on fibrogenic activation and peripheral organ cell apoptosis in experimental ALI.

In the present study, ALI was induced in rats by the administration of paraquat, a herbicide which causes mild lung injury (Rocco et al., 2001) with epithelial cell apoptosis in distal organs (Steimback et al., 2009). The paraquat model of ALI was chosen instead of other models because the lung damage it induces is stable and the level of $PaCO_2$ remained around the normal range, whereas in other models (*e.g.*, endotoxin and surfactant depletion), lung damage usually progresses to hypercapnia, which might have hindered comparison among the NC, HC, and BHC groups. The proportion of alveolar collapse in non-ventilated animals was approximately 30% (Table 2), which is similar to that observed in previous experimental ALI studies (Rocco et al., 2001; Farias et al., 2005; Santiago et al., 2010). Carbon dioxide was increased with an inhaled gas mixture containing 5% CO_2 in air to maintain a $PaCO_2$ between 60 and 70 mmHg, which is defined as a moderate level of hypercapnia (Jung et al., 2013). This method allows increasing $PaCO_2$ without changes in RR or V_T, since these changes may lead to ventilator-induced lung injury, and could add bias to the study design. Inhaled CO_2 is better than low minute ventilation and additional dead space to reduce lung inflammation, due to a more homogeneous CO_2 distribution in the lung parenchyma (Sinclair et al., 2006). Buffering of hypercapnic acidosis was achieved by intravenous injection of sodium bicarbonate (8.4%), which is commonly used in experimental (Sinclair et al., 2002; Abolhassani et al., 2009) and clinical studies (Brower et al., 2004). To evaluate the effects of therapeutic hypercapnia with and without acidosis on fibrogenesis, we measured the lung-tissue expression of PCIII mRNA, as this is the first collagen to be remodeled in the course of lung fibrogenesis (Garcia et al., 2004) and has been used as an early marker of lung parenchyma remodeling (Farias et al., 2005). Finally, animals were ventilated with a tidal volume of 8 ml/kg so as to avoid additional hypercapnia due to lower minute ventilation. A PEEP of 5 cmH_2O was applied to minimize possible interactions

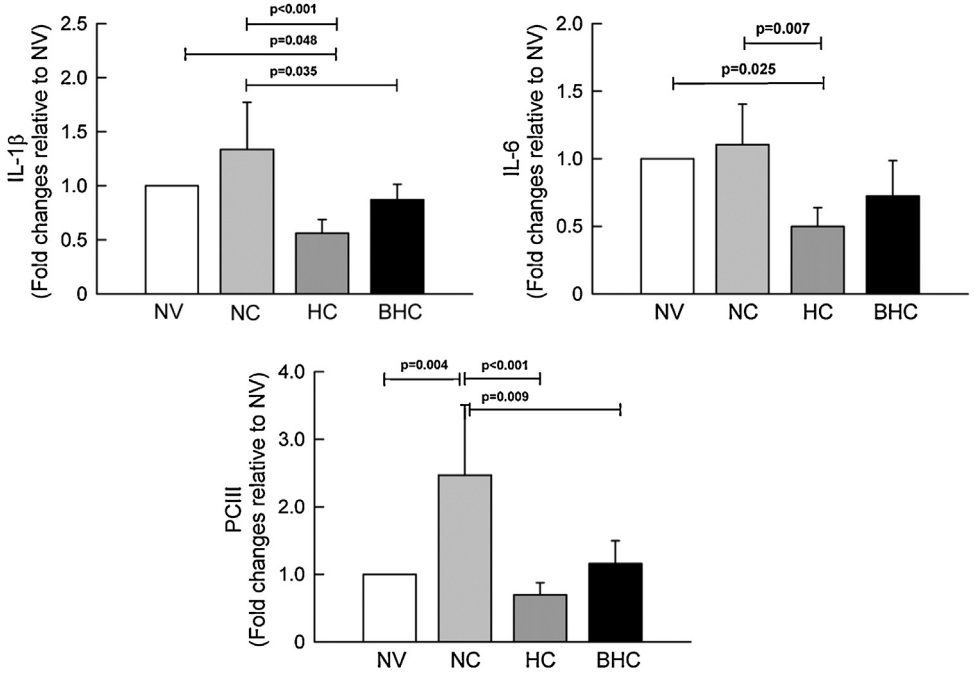

Fig. 2. Real-time PCR analysis of IL-6, IL-1β, and PCIII mRNA expression in rat lung tissue in paraquat-induced ALI rats during different strategies: (a) non-ventilated (NV), (b) normocapnia (NC), (c) hypercapnia (HC), and (d) buffered hypercapnia (BHC). Data are normalized to GAPDH expression. The *y* axis represents fold increase compared with NV. Values expressed as mean (±SD) of five animals in each group. *Significantly different from NV ($p < 0.05$). **Significantly different from NC ($p < 0.05$). #Significantly different from HC ($p < 0.05$).

between mechanical ventilation and ventilator-induced lung injury (Santiago et al., 2010).

In the model of paraquat-induced ALI used in this experiment, hypercapnia, regardless of acidosis, decreased the number of neutrophils in the lung tissue and inflammatory infiltration in alveolar septa compared to NC. Since the experiments lasted only 1 h, we hypothesize that neutrophils may have been reduced due to neutrophil apoptosis or necrosis, which occur earlier than synthesis of pro-inflammatory or anti-inflammatory mediators that affect neutrophil chemoattraction. Controversies remain as to the potential mechanisms underlying the effects of hypercapnia on lung neutrophilic infiltration, which depend on the level of $PaCO_2$, the duration of hypercapnia, and the route of hypercapnia induction (inhalation or mechanical ventilation). Hypercapnic acidosis has been shown to reduce lung neutrophil infiltration in different ALI models, such as the ischemia–reperfusion model in rabbits (Laffey et al., 2000a), intratracheal instillation (Laffey et al., 2004) and intraperitoneal injection (Norozian et al., 2011) of endotoxin, and cecal ligation and puncture (Higgins et al., 2009) in rats. However, it is not clear whether the protective effects of hypercapnic acidosis in these models are neutrophil-dependent. In *E. coli* pneumonia-induced ALI, the protective effects of hypercapnic acidosis were maintained even with neutrophil depletion, suggesting a minor role of neutrophils (Ni Chonghaile et al., 2008). On the other hand, hypercapnic acidosis has been shown to significantly attenuate LPS-induced NF-κB activation by suppression of IκB-alpha degradation, which in turn did not promote neutrophil recruitment into the lung tissue (Takeshita et al., 2003).

Hypercapnia with and without acidosis reduced IL-6 and IL-1β expression in the lung tissue. This is in agreement with a previous *in vitro* study showing that hypercapnia inhibits IL-6 expression irrespective of extracellular and intracellular acidosis (Wang et al., 2010), but in contrast with another *in vitro* study, which reported smaller anti-inflammatory effects with isocapnic acidosis and buffered hypercapnia compared to hypercapnic acidosis (Takeshita et al., 2003). An *in vivo* study showed that infection-induced injury is worsened after renal buffering of hypercapnic acidosis (Nichol et al., 2009). These differences may be explained by the ALI model used and the hypercapnia induction method (Lang et al., 2005).

Hypercapnic acidosis reduced PCIII expression, which may be related to a direct effect of CO_2 on fibroblasts and/or to a reduction in lung inflammation. Additionally, chronic exposure to hypercapnia (Ryu et al., 2010) yielded decreased collagen levels in healthy mice (Ryu et al., 2010), but we cannot directly extrapolate these results to acute hypercapnic acidosis associated or not with ALI.

The reduction in neutrophils and inflammatory mediators in lung tissue induced by hypercapnia led to a greater decrease in alveolar collapse. PaO_2 increased throughout the experiment, but did not differ among groups. Since the animals were ventilated in air, the alveolar PO_2 was probably lower in HC and BHC compared to NC due to an increase in alveolar PCO_2. However, PaO_2 was comparable among groups because the number of opened alveoli increased.

Hypercapnia with or without acidosis led to a reduction in the degree of lung and kidney cell apoptosis. This is in agreement with a previous study showing that hypercapnia decreased lung cell apoptosis in lung ischemia–reperfusion injury (Laffey et al., 2000b). In contrast, *in vitro* studies have reported that hypercapnia does not induce apoptosis in type II alveolar epithelial cell (AECII) culture (Lang et al., 2000; Vaporidi et al., 2005) or increase AECII apoptosis in cells previously exposed to a mixture of cytokines and lipopolysaccharide (Lang et al., 2000).

Hypercapnia did not affect liver cell apoptosis as computed by the TUNEL technique, which may be related to the short duration of the experiment.

5. Limitations

First, paraquat was used to induce ALI. Therefore, we do not know if similar results would be obtained in larger animals and/or other experimental ALI models. Second, the short duration of the experiments, which lasted for only 1 h, precludes the assessment of possible long-term effects of hypercapnia with and without acidosis and the analysis of protein levels. Keeping small animals with ALI and hypercapnic acidosis alive for a longer period of time would require administration of higher amounts of fluids — and, sometimes, vasoactive drugs (*e.g.*, noradrenaline) — to maintain MAP higher than 60 mmHg, and bicarbonate to counteract intense metabolic acidosis. All these therapeutic strategies might interfere with the individual gene activation. Fourth, we used a moderate level of hypercapnia ($PaCO_2$ between 60 and 70 mmHg), and do not know whether higher levels of CO_2 may elicit different results. Fifth, owing to controversies related to the mechanisms underlying the effects of hypercapnia on lung neutrophilic infiltration, further studies are required to clarify this issue. Sixth, our results cannot be directly extrapolated to use of the amino alcohol tromethamine (tris-hydroxymethyl aminomethane) (THAM), which penetrates cells easily and can simultaneously buffer pH changes and reduce the partial pressure of CO_2 (Nahas et al., 1998), but may also induce systemic side effects (Hoste et al., 2005). Seventh, the potential risks for acute hypercapnic acidosis, such as adverse effects on myocardial function, pulmonary vasoconstriction, skeletal muscle, diaphragm, and cerebral blood flow, were not evaluated. Eighth, even though lung water content was not evaluated using the wet/dry lung ratio, a comprehensive histological acute lung injury score was analyzed.

In conclusion, in the current rat model of paraquat-induced ALI, hypercapnia, regardless of acidosis, reduced lung inflammation, expression of PCIII, and lung and kidney cell apoptosis, with no significant changes in liver cell apoptosis.

Competing interest

The authors have no conflict of interest.

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